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Ruminal fermentation of Nellore steers fed crude glycerine replacing starch vs. fibre-based energy ingredient in low or high concentrate diets

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ABSTRACT. Twelve ruminally cannulated steers (401.0 ± 41.5 kg) and 24 mo were used in a replicated arrangement truncated Latin Square with six animals in six treatments and four periods to evaluate the effect of crude glycerine (CG; 80.3% of glycerol) with starch or fiber-based energy ingredients in the concentrate on DMI, DM (DMD) and NDF digestibility (NDFD) and ruminal parameters. Experimental periods were 19 days (14 days for adaptation and 5 days to sampling). Diets were: CO - without CG and corn as ingredient of concentrate; CGC - inclusion of CG (10% of DM) with corn in the concentrate; and CGSH - inclusion of CG (10% of DM) with soybean hulls (SH) in the concentrate. All three diets were offered at low (LC) or high (HC) concentrate level, CL (40 or 60%). Animals fed LC or HC diets had similar DMI, DMD and NDFD. Animals fed diets with CG associated with corn or SH had higher propionate concentrations and lower A:P ratio. Diets with HC increase the propionate but do not affect the NDFD. CG (10% of DM) can be used to replace corn or SH in diets with 40 or 60% of concentrate, without affect NDFD.

Keywords: acetate, glycerol, propionate, pH, soybean hulls.

Fermentação ruminal de novilhos Nelore alimentados com glicerina bruta substituindo amido vs. ingrediente energético a base de fibra em dietas de baixo ou alto concentrado

RESUMO. Doze novilhos canulados no rúmen (401,0 ± 41,5 kg) e 24 meses de idade foram usados em delineamento de quadrado latino truncado replicado com seis animais, seis tratamentos e quatro períodos, para avaliar o efeito da glicerina bruta (GB; 80,3% de glicerol) com amido ou ingredientes energéticos a base de fibra no concentrado sobre o CMS, digestibilidade da MS (DMS) e FDN (DFDN) e parâmetros ruminais. O período experimental foi 19 dias (14 dias de adaptação e 5 dias de coleta). As dietas foram: CO – sem glicerina bruta e milho como ingrediente do concentrado; GBM – GB (10% da MS) com milho no concentrado; GBCS - GB (10% da MS) com casca de soja no concentrado. As três dietas foram ofertadas em baixo (BC) ou alto (AC) teor de concentrado (TC; 40 ou 60%). Animais alimentados com BC ou AC apresentaram similar CMS, DMS e DFDN. Animais alimentados com GB com milho ou CS apresentaram maior concentração de propionato e menor relação A:P. Dietas com AC aumentou a concentração de propionato mas não afetou a DFDN. GB (10% da MS) pode ser usada para substituir o milho ou CS em dietas com 40 ou 60% de concentrado, sem afetar a DFDN.

Palavras-chave: acetato, glicerol, propionato, pH, casca de soja.

Introduction

Glycerol is a byproduct from the biodiesel agroindustry and has been used as an energy source in diets of ruminants (Donkin, Koser, White, Doane, & Cecava, 2009; Eiras et al., 2013; Hales et al., 2013; Meale, Chaves, Ding, Bush, & McAllister, 2013; Cruz et al., 2014; Eiras et al., 2014). A reduction in NDF digestibility has frequently been reported from the inclusion of glycerine in ruminant diets (Donkin et al., 2009) apparently from a growth inhibition of cellulolytic bacteria. However, dietary glycerine appears to have a differential effect on fiber digestion depending on the level of dietary starch. Concerns about reduction of fiber digestibility associated with feeding glycerine are limited in feedlot cattle fed high-concentrate finishing diets because fiber concentrations are normally low (Hales et al., 2013).

Propiogenesis has been shown to substantially increase when glycerol is added to high-fiber diets in contrast to when added to high-starch diets incubated *in vitro* (Rémond, Souday, & Jouany, 1993). Thus, the addition of glycerol could improve the energetic efficiency of growing ruminants fed high forage diets more so than for those fed with a greater proportion of concentrate in the diets (Farias et al., 2012; Eiras et al., 2014). The objective of this study was to evaluate the effects of crude glycerine on diet digestibility and ruminal fermentation when fed at two levels of concentrate to Nellore steers.

Materials and methods

The trial was realized at the animal facilities of the Department of Animal Science, Universidade Estadual Paulista, Jaboticabal, São Paulo, Brazil, and followed the humane animal care and handling procedures set at the institution. Twelve ruminally cannulated Nellore steers with an average BW of 401.0 ± 41.5 kg and 24 months of age were used in a replicated, truncated Latin square design. Each truncated square consisted of six animals, six treatments and four periods, set to evaluate treatment effects on intake, apparent total tract digestibility, ruminal pH, ammonia-N concentration and VFA of steers fed in finishing phase. Animals were treated for internal and external parasites at the beginning of the experiment and kept in individual pens of approximately 21 m^2 with protected feeders and waterers throughout the adaptation and sampling periods. Experimental periods were 19 days in total, with the first 14 days for adaptation to the diet and the last 5 days used for sampling. Dry matter intake was measured over the 5 days of sampling, while feces were sampled for 3 days and liquid and ruminal contents over a single day.

The treatment design was a 3 x 2 factorial with the first factor consisting of 3 combinations of level of glycerine and main substrate in the concentrate, while the second factor consisted of 2 proportions of concentrate in the total diet. The 3 glycerine/substrate diets were as follow: CO – 0% crude glycerine with corn as the main ingredient in the concentrate; CGC - crude glycerine at 10% of DM with corn as the main ingredient in the concentrate; and CGSH - crude glycerine at 10% of DM with soybean hulls as the main ingredient in the concentrate. These three diets were offered at two levels of concentrate: 40 and 60% of DM.

Crude glycerine was sourced from a soybean oil-based biodiesel production company, (ADM, Rondonópolis, Brazil) and contained 80.3 glycerol, 1.6 ether extracts, 5.0 ash and 12.0% water. Corn silage was used as the only source of roughage. Concentrates were made of ground corn, soybean hulls, soybean meal, urea/ammonium sulfate and a mineral mixture (Table 1). Soybean meal was used as the primary source of protein in all diets. A mixture of urea and ammonium sulfate was used to make the diets nearly isonitrogenous at approximately 14.4 ± 0. 2% CP (Valadares Filho, 2006). Concentrate recipes and their chemical compositions are reported in Table 1.

Table 1. Diet composition (DM basis)[1].

Item	40% concentrate			60% concentrate		
	CO	CGC	CGSH	CO	CGC	CGSH
Ingredient, % of DM						
Corn silage	60.0	60.0	60.0	40.0	40.0	40.0
Corn	26.3	16.0	-	46.4	36.1	-
Soybean meal	10.0	10.0	10.0	10.0	10.0	10.0
Soybean hulls	-	-	16.2	-	-	36.6
Crude glycerine	-	10.0	10.0	-	10.0	10.0
Urea/ammonium sulfate	0.70	1.00	0.80	0.60	0.90	0.40
Mineral premix[2]	3.0	3.0	3.0	3.0	3.0	3.0
Nutrient composition, %						
DM	57.6	57.9	58.6	67.1	67.4	68.9
CP	14.3	14.7	14.6	14.5	14.2	14.1
NDF	30.1	28.5	37.6	25.2	23.6	43.0
EE[3]	3.13	3.01	2.92	3.18	3.05	2.83
NFC[4]	47.0	49.5	40.1	53.9	55.5	35.5
ME, Mca L kg^{-1}	2.56	2.54	2.50	2.68	2.67	2.60

[1]CO = corn, without crude glycerine; CGC = 10% crude glycerine with corn; CGSH = 10% crude glycerine with soybean hulls. [2]Mineral mixed contained per kg: Ca, 210 g; P, 20 g; S, 37 g; Na, 80 g; Cu, 490 mg; Mn, 1.42 mg; Zn, 1.83 mg; I, 36 mg; Co, 29 mg; Se, 9 mg; F max., 333 mg. [3]Ether extract. [4]Non fiber carbohydrates = 100 – (CP + EE + Ash + NDF).

Diets were fed as a total mixed ration in which corn silage and concentrate (previously mixed) were weighed and thoroughly mixed before each feeding. Cattle were fed once daily at 0700 hour, and feed refusals were sampled and weighed daily for each individual pen. Amounts of feed offered daily to animals were calculated according to previous DMI and adjustments were made daily to ensure *ad libitum* intake (5-10% refusal). Steers had free access to water throughout the trial. Samples of diets (forage and concentrate) and orts from each animal were collected throughout the 5 days of the sampling period (days 15 to 19), then composited by animal and period for subsequent analyses. Dry matter intake and NDF intake were calculated as the difference between the amounts offered and refused based on the chemical analysis of the composited sample for each steer within each period. Feed samples and orts were frozen at -18°C until analyzed for DM [method 934.01; AOAC (2005], ether extract Association Official Analytical Chemist (AOAC, 2005), N Leco Instruments Inc., method 976.06, AOAC (2005] and multiplied by 6.25 to obtain CP, ash [method 924.05; AOAC (2005)], while NDF was determined by the method of Van Soest, Robertson, and Lewis (1991) using Ankon bags (Ankon Technology Corp., Fairport, NY, USA).

Total fecal collections were done over 3 days between days 15 and 17 of each period. Feces were collected immediately after each spontaneous defecation, and stored in 20 L buckets. At the end of each 24 hours collection, feces from each bucket were weighed, manually mixed, and daily aliquots (approximately 300 g) were collected, ground in a Wiley mill (1 mm screen; model MA680, Marconi, Piracicaba, SP) and dried in a forced-air oven at 60°C for 72 to 96 hours. At the end of each period, 10 g of each of the 3 dried samples from each animal were used to prepare a composite sample for each animal-period. All composite samples were stored in sealed plastic flasks for subsequent analysis of DM and NDF.

Rumen pH, ammonia-N, and VFA were measured on samples taken over a 12 hour span on day 18 of each experimental period. Ruminal content was obtained at 0, 2, 4, 6, 8, 10 and 12 hour after the 0700 hour feeding. Samples of rumen samples content were obtained from several sites within the rumen and were subsequently strained through 2 layers of cheesecloth. Ruminal pH was immediately measured on the separated liquid using an electric pH meter (Nova Técnica, PHM, Piracicaba, State São Paulo). Immediately the pH measurements, samples were poured into 50 mL plastic flasks containing 1 mL of 9.3 *M* H_2SO_4 and frozen at -20°C until subsequent. Ruminal fluid NH_3 concentration was determined by distillation with 2 MKOH in a micro-Kjeldahl system according to the original procedures of Fenner (1965). Sub-samples of rumen fluids were centrifuged at 13000 x g (4°C) for 30 min and VFA was quantified by gas chromatography (GC Shimatzu model 20-10, automatic injection) using capilar column (SP-2560, 100 m × 0.25 mm of diameter and 0.02 mm of thickness, Supelco, Bellefonte, PA) according to the method of Palmquist and Conrad (1971).

Data were analyzed as linear mixed models using the MIXED procedure of SAS V9.2 (SAS Inst. Inc., Cary, NC). For variables measured only once on each animal-period, the model included the fixed effects of diet type (2 df), concentrate level (1 df) and their interaction (2 df), and the random effects of animals (5 df), periods (3 df) and a residual error (i.e., the animal x period x diet interaction, 10 df). Variables with repeated measurements on each animal-period (i.e, pH, ammonia-N and VFA) were analyzed using a model with the same effects previously stated with the additional fixed effects of hourly times (6 df), time x diet type (12 df), time x concentrate level (6 df), time x diet x concentrate level (12 df) and a random residual effect (i.e., the animal x period x diet x time, 108 df). Several covariance structures were tested to account for the correlation of errors due to the repeated measures. The structure resulting in the smallest Schwarz Bayesian criteria (BIC) was considered the most appropriate in the final model used for testing fixed effects. Differences between least-squares means were tested ($p < 0.05$) using Tukey's test.

Results and discussion

Level of concentrate in the diet had no effect on DMI ($p = 0.64$), DM digestibility ($p = 0.85$) and NDF digestibility ($p = 0.61$; Table 2). The negative effect of high concentrate diets on DMI can occur due to a depression in fiber digestibility. Sarwar, Firkins, and Eastridge (1992) reported that the extent of digestion of NDF decreased as the proportion of concentrate in the diet increased. However, in the present study DM and NDF digestibility did not differ between two levels of concentrate used and negative effects on DMI were not observed, possibly because the reduced feed intake frequently reported in the literature generally occurs at concentrate levels exceeding 70 to 75% concentrate (Tremere, Merrill, & Loosli, 1968); whereas the maximum concentrate level used in this study was 60% of diet DM.

Likewise, the effect of diet 10 vs.0% or corn vs. hulls had no significant effect on DMI, DM digestibility and NDF digestibility ($p > 0.05$; Table 2).

Table 2. Effect of crude glycerine associated with corn or soybean hulls in two levels of concentrate on DMI and nutrient digestibility.

Item	40% of concentrate			60% of concentrate			SEM	*P*-value[2]				
	CO	CGC	CGSH	CO	CGC	CGSH		CL	Diet 10 vs. 0%	Diet corn vs. hulls	CL by diet 10 vs. 0%	CL by diet corn vs. hulls
DMI, kg day^{-1}	6.53	7.41	6.73	7.85	6.64	6.65	0.51	0.67	0.37	0.42	0.02	0.41
DMD, %[3]	51.86	59.46	55.79	59.28	57.27	52.15	4.00	0.89	0.84	0.23	0.16	0.84
NDFD, %[4]	34.85	40.94	41.76	42.94	42.63	39.25	6.72	0.73	0.63	0.82	0.51	0.74

[1]CO = corn, without crude glycerine; CGC = crude glycerine associated with corn; CGSH = crude glycerine associated with soybean hulls. [2]CL = concentrate level effect; Diet 10 vs. 0% = contrast is for 10 glycerol/corn and 10% glycerol/hulls vs. 0% glycerol; Diet corn vs. hulls = contrast is for 10 glycerol/corn vs. 10% glycerol/hulls; CL by diet 10 vs. 0% = contrast is for 10 glycerol vs. 0% glycerol at 40 CL vs. 60%; CL by diet corn vs. hulls = contrast is for 10 glycerol/corn vs 10% glycerol/hulls at 40 CL vs. 60%; [3]Dry matter digestibility; [4]Neutral detergent fiber digestibility.

Although some studies have reported a negative effect of feeding glycerine fiber digestibility in ruminants (Abo El-Nor, AbuGhazaleh, Potu, Hastings, & Khattab, 2010; Shin, Wang, Kim, Adesogan, & Staples, 2012), results from other studies suggest that crude glycerine can be included at levels up to 10% of the DM, replacing rapidly fermentable starch in finishing beef cattle diets without affecting intake or nutrient digestibility (Ramos & Kerley, 2012; Bartoň et al., 2013). The lack of digestibility depression from a starch-based diet with 10% glycerine in our study is in agreement with these reports.

In the present study, we hypothesized that the inclusion of crude glycerine in combination with a fiber-based energy ingredient in the concentrate would decrease NDF digestibility and, consequently DMI. Soybean hulls have 66.3% of NDF concentration in DM basis (National Research Council [NRC], 2000). However, the soybean hulls has a small feed particle size (Mertens, 1997) and could result in a more rapid ruminal escape and in a reduction of the ruminal fill (Iraira et al., 2013). Although the soybean hulls have high NDF content and the experimental diets which crude glycerine replaced soybean hulls increases NDF concentration (Table 1), the digestibility was not affected in these diets because the soybean hulls had lower time to retention in the rumen.

There was an interaction of CL by diet 10 vs. 0% on DMI ($p = 0.02$). As observed in our study, the intake has been variable and generally depressed in studies where crude glycerine replaced rapidly fermentable starch in diets with high level of concentrate (Parsons, Shelor, & Drouillard, 2009; Avila-Stagno et al., 2013; Meale et al., 2013).

The ruminal pH was lower to animals fed high concentrate diets than ruminal pH from animals fed low concentrate diets ($p < 0.01$; Table 3; Figure 1). The DMI is a major determinant of rumen pH and individual animal difference in rumen pH depends on the capacity of the animal to buffer and to absorb organic acids produced in the rumen which determines the drop of rumen pH after feeding large amounts of fermentable carbohydrates (Krause & Oetzel, 2006). The DMI from animals fed with different concentrate levels (40 or 60%) was similar among these diets, but generally, increasing the grain content of the diet usually results in a decline in rumen pH as a result of an increase in the supply of rapidly fermentable carbohydrates (Van Kessel & Russell, 1996; Lana, Russell, & Van Amburgh, 1998; Walsh et al., 2009).

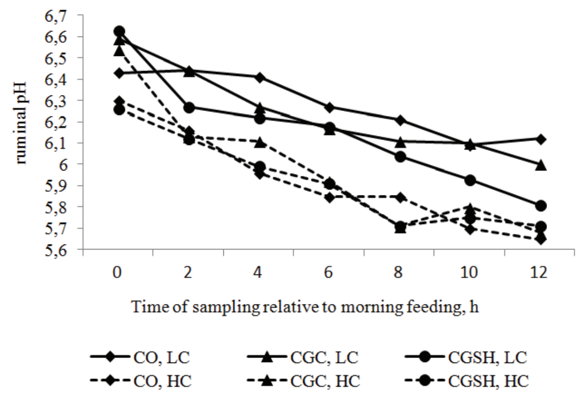

Figure 1. pH in the ruminal fluid of steers fed diets CO = corn, without crude glycerine; CGC = crude glycerine associated with corn; CGSH = crude glycerine associated with soybean hulls at two levels of concentrate. Significant effects of concentrate level ($p < 0.01$) were detected.

Some researchers have reported that ruminal pH decreases when crude glycerine replaces starch in diets to ruminants (Mach, Bach, & Devant, 2009; Ramos & Kerley, 2012), because the ruminal fermentation of glycerol is faster than starch. Contrary to these experiments, no differences were detected in pH, in the present study, when crude glycerine replaced corn or soybean hulls in 10% of diet DM. Likewise, DeFrain, Hippen, Kalscheur, and Jardon (2004) reported no differences in pH when crude glycerine replaced corn starch in diets to ruminants.

Differences in ammonia-N concentration was not observed in animals fed low or high concentrate diets ($p = 0.14$; Table 3); however, significant effect of diet 10 vs. 0% ($p = 0.03$) and diet corn vs. hulls ($p = 0.01$) was detected (Figure 2). Animals fed crude glycerine associated with corn or soybean hulls had lower ammonia-N concentration than animals fed diets without crude glycerine. The suppressed microbial fermentation activity can be induced by ruminal transit (Cole & Hutcheson, 1985) and the crude glycerine is readily absorbed through the rumen wall or fermented to propionate.

Thus, due to the fast disappearance of crude glycerine from the rumen, it can contribute to decrease the ammonia-N concentration in the rumen. Likewise, Avila-Stagno et al. (2013) showed a linear decrease of ammonia-N concentration in the rumen when increase the crude glycerine concentration up to 150 g kg^{-1} of the diet.

Animals fed diets with crude glycerine associated with soybean hulls had lower ammonia-N concentration than animals fed diets with crude glycerine associated with corn. The soybean hulls exhibit a greater rate of ruminal passage than corn (Ipharraguerre & Clark, 2003), remaining less time at the rumen, reducing the microbial fermentation activity and favoring the lower production of ammonia-N in the rumen.

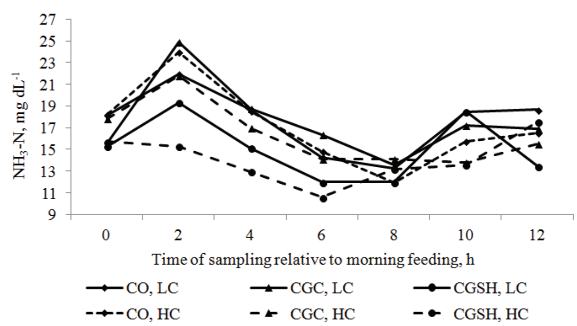

Figure 2. Ammonia-N concentration in the ruminal fluid of steers fed diets CO = corn, without crude glycerine; CGC = crude glycerine associated with corn; CGSH = crude glycerine associated with soybean hulls at two levels of concentrate. Significant effects of diets ($p < 0.05$) were detected.

There was an interaction of CL by diet 10% vs. 0% ($p = 0.04$; Table3; Figure 3) on acetate concentrations. Acetate concentrations were higher in ruminal fluid from animals fed diets with low concentrate without crude glycerine than diets with high concentrate without crude glycerine. In diets with high proportion of roughage, the acetate concentrations are greater because increase the quantity of fiber fermented.

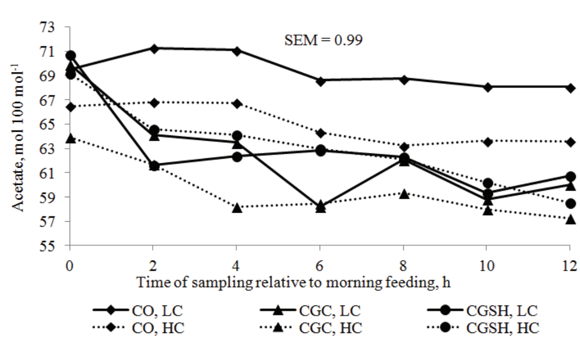

Figure 3. Acetate concentrations in the ruminal fluid of steers fed diets CO = corn, without crude glycerine; CGC = crude glycerine associated with corn; CGSH = crude glycerine associated with soybean hulls at two levels of concentrate. There was an interaction between concentrate level x diets ($p < 0.05$).

We hypothesized that animals fed diets with crude glycerine associated with corn or soybean hulls in low concentrate diets may produce lower acetate concentrations than animals fed diets with crude glycerine associated with corn or soybean hulls in high concentrate diets because crude glycerine may produce others VFA more efficiently at low-concentrate diets.

The decrease in acetate concentrations were observed in animals fed crude glycerine associated with starch- or fiber-based ingredients in low concentrate than animals fed low concentrate diets without crude glycerine. Although the acetate concentrations from animals fed crude glycerine associated with starch- or fiber-based ingredients in low concentrate were not lower than acetate concentrations from animals fed high concentrate diets, these concentrations were similar between these diets, reporting the glycogenic property of glycerol in low concentrate diets.

Starch fermentation in the rumen yields more propionate, less acetate production, unlike the soybean hulls that are low in lignin and composed of a large proportion of potentially digestible fiber (Quicke, Bentley, Scott, Johnson, & Moxon, 1959; Hsu et al., 1987).

Therefore, when soybean hulls are fermented in the rumen, more acetate than propionate is produced. Evaluating diets with crude glycerine associated with corn or soybean hulls (low or high concentrate), was observed that acetate concentrations were similar in animals fed crude glycerine associated with corn or soybean hulls both in low concentrate diets, but it is not observed in high concentrate diets, which acetate concentrations were higher in diets with crude glycerine associated soybean hulls than diets with crude glycerine associated with corn.

Despite these results, can be observed that crude glycerine had a greater efficiency to reduce the acetate concentrations associated with fiber-based ingredients in low concentrate, because similar results did not achieved in high concentrate diets.

There was a significant effect of CL ($p < 0.01$) and diet 10 vs. 0% ($p < 0.01$) on propionate concentrations (Table 3; Figure 4).

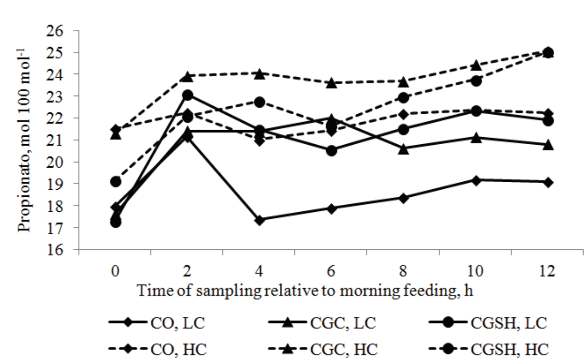

Figure 4. Propionate concentration in the ruminal fluid of steers fed diets CO = corn, without crude glycerine; CGC = crude glycerine associated with corn; CGSH = crude glycerine associated with soybean hulls at two levels of concentrate. Significant effects of concentrate level ($p < 0.01$) and diets ($p < 0.01$) were detected.

Table 3. Effect of crude glycerine associated with corn or soybean hulls in two levels of concentrate on pH, ammonia-N and VFA concentrations.

Item	40% of concentrate			60% of concentrate				P-value[2]				
	CO	CGC	CGSH	CO	CGC	CGSH	SEM	CL	Diet 10 vs. 0%	Diet corn vs. hulls	CL by diet 10 vs. 0%	CL by diet corn vs. hulls
pH	6.28	6.24	6.16	5.93	5.99	5.92	0.09	<0.01	0.40	0.05	0.11	0.80
NH_3-N (MG dL^{-1})	17.63	17.62	15.10	17.09	16.29	14.13	0.79	0.14	0.03	0.01	0.76	0.76
Acetate	69.36	62.41	62.86	64.98	59.55	63.12	0.99	0.01	<0.01	0.01	0.04	0.06
Propionate	18.71	20.71	21.16	21.86	23.72	22.50	1.08	<0.01	<0.01	0.37	0.22	0.06
Butyrate	13.90	19.21	17.47	14.90	18.03	16.14	0.83	0.15	<0.01	<0.01	0.01	0.84
A:P ratio[3]	3.67	2.99	3.04	3.04	2.56	2.89	0.15	<0.01	<0.01	0.02	0.02	0.09

[1]CO = corn, without crude glycerine; CGC = crude glycerine associated with corn; CGSH = crude glycerine associated with soybean hulls. [2]CL = concentrate level effect; Diet 10 vs. 0% = contrast is for 10 glycerol/corn and 10% glycerol/hulls vs. 0% glycerol; Diet corn vs. hulls = contrast is for 10 glycerol/corn vs. 10% glycerol/hulls; CL by diet 10 vs. 0% = contrast is for 10 glycerol vs. 0% glycerol at 40 CL vs. 60%; CL by diet corn vs. hulls = contrast is for 10 glycerol/corn *vs.* 10% glycerol/hulls at 40 CL vs. 60%; [3]Acetate:propionate ratio.

Animals fed high concentrate diets had greater propionate concentrations than animals fed low concentrate diets. When propionate concentrations increase in ruminal fluid, the pH decreases (Ørskov, 1986). Linear decrease in the molar proportion of acetic acid and simultaneous increase in propionic acid concentration occurs in response to increasing the grain content (McGeough et al., 2010). Likewise, in the present study, the propionate concentrations increase and the pH were reduced in ruminal fluid from animals fed high concentrate diets. It has also been well established (Moe & Tyrrell, 1979; Johnson & Johnson, 1995) that altering the dietary forage-to-concentrate ratio, specifically the fiber to-starch ratio, affects the proportion of the individual VFA in the rumen.

The propionate concentrations ($p < 0.05$) and A:P ratio ($p < 0.05$; Table 3; Figure 6) were affected by inclusion of crude glycerine in diets replacing starch- or fiber-based energy ingredients in the concentrate. animals fed diets with crude glycerine associated with corn or soybean hulls had higher propionate concentrations and lower A:P ratio in ruminal fluid than animals fed diets without crude glycerine. The increase in molar proportion of propionate resulted in reduction of acetate:propionate ratio, according with Lee et al. (2011) that reported the propionate as an mainly end product from fermentation of glycerol. The increase of propionate concentrations by feeding with glycerol has been reported when crude glycerine replaced roughage (Hales et al., 2013) or rapidly fermentable starch in the concentrates (Avila et al., 2011; Ramos & Kerley, 2012), confirming the glycogenic properties of glycerol.

Effects of glycerol on ruminal fermentation are a shift in VFA production in favor of propionate, with an even greater increase in butyrate at the expense of acetate both in vitro and in vivo (Rémond et al., 1993). In this work, there was an interaction of CL by diet 10 vs. 0% ($p = 0.01$) on butyrate concentrations.

Butyrate concentrations were greater in diets with crude glycerine associated with corn or soybean hulls than diets without crude glycerine (Table 3; Figure 5) in different levels of concentrate. Glycerol is metabolized by *Megasphaera elsdenii*, *Streptococcus bovis*, and *Selenomonas ruminantium* (Stewart, Flint, & Bryant, 1997), and *Megasphaera elsdenii* has been associated with increases in butyric acid in ruminal fluid (Hales et al., 2013). The increase in the molar proportion of butyrate with glycerol substitution level is consistent with the findings of others (Rémond et al., 1993; Wang et al., 2009; Abo El-Nor et al., 2010) who reported that the molar proportion of butyrate in the VFA mixture increased when glycerol was added in the diets.

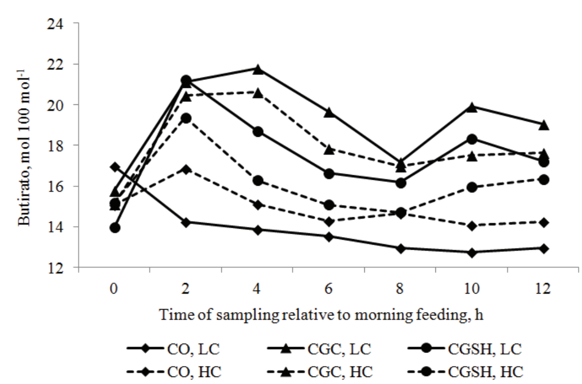

Figure 5. Butyrate concentrations in the ruminal fluid of steers fed diets CO = corn, without crude glycerine; CGC = crude glycerine associated with corn; CGSH = crude glycerine associated with soybean hulls at two levels of concentrate. There was an interaction between concentrate level x diets ($p < 0.05$).

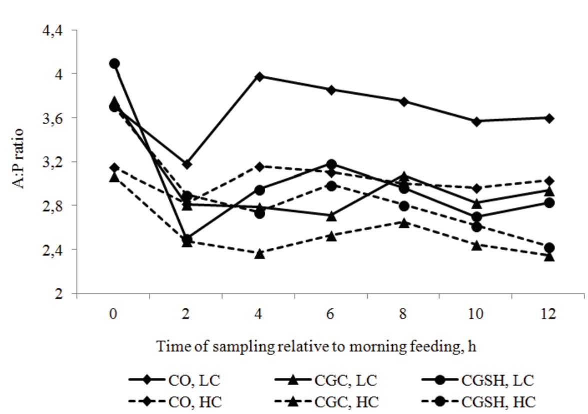

Figure 6. Acetate: propionate ratio in the ruminal fluid of steers fed diets CO = corn, without crude glycerine; CGC = crude glycerine associated with corn; CGSH = crude glycerine associated with soybean hulls at two levels of concentrate. There was an interaction between concentrate level x diets ($p < 0.05$).

Conclusion

Increasing concentrate content (40 to 60%) reduces the pH, increases the propionate but does not affect the NDF digestibility of diets and DMI by animals. Crude glycerine can be used to replace corn or soybean hulls in 10% of diet DM in diets with 40 or 60% of concentrate, without affect NDF digestibility. Moreover, the inclusion of crude glycerine in diets associated with starch- or fiber-based energy ingredients increases butyrate concentrations and reduces the acetate:propionate ratio as a result of increases of propionate concentrations.

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